

## **REMARKS**

Pursuant to the Restriction Requirement, Applicants hereby elect the invention of Group I, claims 1-7 and 12-15, with traverse.

The Examiner mentions that Groups I-IV lack the same or corresponding special technical features for the following reasons. The present invention of Groups I-IV have common technical feature (i.e., method of assigning signals in NMR spectra which is disclosed in US 6,111,066).

However, the method of assigning signals in NMR spectra according to the present invention is different from the method of assigning signals in NMR spectra which is disclosed in US Patent 6,111,066. Therefore, the present invention of Groups I-IV has the same or corresponding special technical features and fulfills unity of invention. The details are mentioned below.

### **(1) Method of assigning signals in NMR spectra according to the present invention**

In the method of assigning signals in NMR spectra according to the present invention, assignment of all signals can be carried out by preparing only approximately 20 types of labeled proteins. Therefore, assignment of signals can be simply carried out by using a small amount of protein in a short time without a high level of analysis skill.

In claim 1, a protein is prepared as follows:

- (i) the carbon atom(s) at position 2 and/or at position 1 and the nitrogen atom at position 2 of either one of the amino acids adjacent to an amino acid to be identified on the amino acid sequence of the protein are double-labeled for the NMR measurement,  
and
- (i-2) any one of the nitrogen, carbon, and hydrogen atoms at position 2 of at least the amino acid to be identified are labeled for the NMR measurement.

Then, the NMR measurement is performed on the above-described protein as follows:

- (ii) NMR measurement is performed to obtain the correlation signal of the amide proton of an amino acid residue to be identified, adjacent to the double-labeled amino acid, with the labeled atom, and

(iii) the signal is compared with the correlation signal of the amide proton of an amino acid residue to be identified with the labeled atom, which is obtained by NMR measurement of a protein wherein any one of the nitrogen, carbon, and hydrogen atoms at position 2 of the amino acid to be identified has been labeled, so as to determine the assignment of the signal of the amino acid to be identified.

(2) Difference between the method of US Patent 6,111,066 and the method of the present invention

The invention of US Patent 6,111,066 aims at improving NMR spectroscopic techniques by (i) increasing the resolution of key signals in the NMR spectrum and (ii) eliminating the splitting of the key signals by an adjacent NMR active nucleus. The means for achieving this object is as recited in Claim 1. A protein is prepared wherein the amino group at  $\alpha$ -position of an amino acid of interest is labeled with  $^{15}\text{N}$ , the carbon atom at the  $\beta$ -position and the carbon atom at the  $\alpha$ -position are labeled with  $^{13}\text{C}$ , and the side chain is not labeled. Then, assignment of the NMR signals is carried out by a combination of HNCA measurement and HN(CO)CA measurement.

As described above, the labeling of protein used in the NMR measurement for the method of assigning signals in NMR spectra according to the present invention is carried on specific carbon atoms, nitrogen atoms and hydrogen atoms in an amino acid to be identified and amino acids adjacent thereto. On the other hand, US Patent 6,111,066 does not disclose such specific labeling method. Therefore, the claimed invention is different from US Patent 6,111,066. Further, in the claimed invention, NMR measurement is carried out to obtain each of the correlation signals for the proteins wherein the aforementioned two specific atoms are labeled, and the thus obtained two signals are compared with each other so as to determine the assignment of the signal of the amino acid to be identified. However, US Patent 6,111,066 does not disclose such NMR measurements. Therefore, the present invention is different from US Patent 6,111,066.

In view of this election and the above remarks, the cited reference does not render moot the special technical feature of the claimed invention. Thus, withdrawal of the Restriction Requirement and a full examination on the merits of the present application is respectfully requested.

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